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Original Article

ASSAY OF ALPHA AND BETA ESTERASE ISOZYMES DURING DIFFERENT DEVELOPMENTAL STAGES OF NEW BREEDING LINES AND RACES OF *BOMBYX MORI* L

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ABSTRACT

Knowledge on enzyme studies in new breeding lines and races of silkworm especially Bombyx mori L is very limited. Hence, in the current study we aimed to evaluate the alpha and beta esterase isozymes quantitatively during different developmental stages of new breeding lines and races viz. Kalimpong-A (KA), B_{18}, Pure Mysore (PM), evolved R_1 and R_2 of Bombyx mori L. Quantitative estimations of in Alpha- and Beta- esterases are expressed in terms of enzyme activity. Alpha- and Beta- esterase activities during the different developmental stages of KA, NB_{18}, PM, evolved R_1& R_2 races were determined using alpha- and beta-naphthyl acetate as substrates. Results inferred that each enzyme is present in a very low concentration in the eggs of all the species studied. Esterase activity increases during larval instars and reaches a peak in pupae stage. It was found to be less in case of adults. The concentration was found to be high in KA followed by R_2, NB_{18}, R_1 and PM. In conclusion, alpha- and beta- esterases activity was found to be highest in the female pupae, and it was found to be less in case of adults. High esterase activity is noticed in pure races than the isolated lines. The esterase activity is high in pupal stage followed by larval stage.

KEYWORDS: Bombyx Mori L, Alpha Esterase, Beta Esterase, Bivoltine, Kalimpong-A (KA), Pure Mysore (PM)

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INTRODUCTION

Gene enzyme systems in Drosophila and a few other animals drew the attention of several workers and the early work on biochemical basis of eye colour mutant in Drosophila, notably by during 1930's, played a crucial role in establishing the relationships between genes and enzymes (Dickinson and Sullivan, 1975).

Every change in the enzyme pattern during development can be directly determined by genetic function. This reflects the change in the metabolism of the developing organism (Pasteur, 1971). Analyzes of different genetic enzyme systems have shown different regulation of genetic activation during metabolic changes. Isozymes can be viewed as ideal gene products for studying gene expression patterns during development. They provide a potential tool and biochemical index to assess genetic variability in natural populations (Ayala *et al.*, 1974a, Ayala *et al.*, 1974b, McKenzie *et al.*, 1974, Narang, 1980). These studies have often been used to follow evolutionary variations in different enzyme patterns, as well as when comparing species (Dickinson and Sullivan, 1975). At the same time, analyzes of isozyme differentiation systems during ontogenesis are of great biological value. The

isoenzymes present during the different stages of development can be studied in two ways. One is to understand the different effects of genes during development and the other is to compare the manifestation of homologous enzymes during the stages of development.

Shyamala et al studied the nature and extent of the influence of chloromycetin on larval digestion and utilization of proteins, fats and minerals in the diet of silkworm *Bombyx mori* L (Shyamala *et al.*, 1959). Ito and Tanaka studied nutritive effect of varying proteins on the growth and development of silkworm *Bombyx mori* L (Ito and Tanaka, 1962). Ito and Mukaiyama investigated the interrelationship between the quantity of dietary protein and the xanthine oxidase activity of larval tissues of silkworm *Bombyx mori*. The enzyme activity increases in fat body, midgut and malpigia tubules with the increase of soyabean meal (Ito and Mukaiyama, 1964). Shimura et al studied enzymatic aspects of protein in synthesis in the polysomes of silk glands (Shimura *et al.*, 1967). Shigematsu investigated the protein metabolism in fat body of silk worm *B. mori* (Shigematsu 1960). Saito studied the rate of metabolism of blood sugar trehalose in the fifth instar larvae of silkworm *Bombyx mori*. It has been shown that on starvation, the rate of disappearance of glycogen in the fat body is more rapid than that of body fluid trehalose (Saito 1959, 1963)

However, knowledge on enzyme studies in new breeding lines and races of silkworm especially *Bombyx mori* L is very limited indicating the type and amount of work to be done in future. At the same time, it is interesting to note that different silkworm races reared in laboratory offer an important testing ground for the application of biochemical methods to taxonomic problems. With this viewpoint, in the current study we aimed to evaluate the alpha and beta esterase isozymes quantitatively during different developmental stages of new breeding lines and races of *Bombyx mori* L.

MATERIALS AND METHODS

Silkworm Varieties and Rearing

The pure races of bivoltine Kalimpong-A (KA) spinning oval white cocoons, New Bivoltine-18 (NB_{18}) spinning dumbbell white cocoons and multivoltine Pure Mysore (PM) spinning pointed yellow cocoons of mulberry silkworm Bombyx *mori* L. were selected for the present breeding programme. These races were obtained from their respective seed areas and are reared in cytogenetics laboratory, Jnana Bharathi, Bangalore University.

The disease free layings were prepared as described by [9], and were incubated at 25°C and relative humidity of 60-70%. On 8th day composite layings were prepared (10-20 layings were prepared 100-200 eggs were collected from each laying). The hatched worms were reared according to the method described by Krishnaswamy (1978). MS variety of mulberry leaves were used in rearing. The worms were reared in mass up to III instar, after III moult 300 worms were collected in three replicates in order to evaluate the rearing performance. Standard temperature and humidity were maintained in the rearing house.

Breeding

Single and three way crosses were made by using the above said three races. The first single cross involved KA females and PM males. The second single cross involved NB_{18} females and PM males. During the course of breeding selection was made at the egg, larva, pupa and cocoon stages to fix the desirable traits. F_5 progenies of the respective crosses were back crossed to their respective bivoltine males to improve commercial characters.

Evolutions of New Lines R_1 and R_2

Females of KA and NB_{18} were crossed with males of PM. The composite layings of F_1 hybrid were brushed and reared under standard laboratory conditions. The selection parameters explained earlier were applied to choose the seed cocoons for the preparation of F_2 layings. The replicates showing higher pupation rate were selected for intra family selection of cocoons. Further, segregation with respect to cocoon colour and built was noticed. Only white oval in case of KAxPM and dumbbell white in case of NB_{18}xPM qualifying the parameter of selection was chosen for breeding in subsequent generations. The females of F_5 were backcrossed to the males of KA and NB_{18} respectively in both the lines and reared up to 11 generations. At the end of the 11^{th} generation the lines R_1 and R_2 were extracted with higher ERR than their respective better parents, with shorter larval period and with moderate cocoon productivity character in case of R_1 and R_2

Table 1

Breeding Plans I and II													
				I							**II**		
	KA	O	O	x	PM	Cto		NB18	O	O	x	PM	Cfo
		+	i-						+	+			
				F1							F1		
				F2							F2		
				F3							F3		
				F4							F4		
F5	x	KA	O	↘	er'		F5x	NB18	Cta	+			
				F1							F1		
				F2							F2		
				F3							F3		
				F4							F4		
				F5							F5		
				F6	(R1)						F6	(R2)	

Preparation of Enzyme Extract

The different developmental stages such as 1st day, 5^{th} day and 9th day eggs, five larval instars (I, II, III, IV, and V instars), early, middle and late stages of male and female pupae, male moths before and after copulation. Female moths before and after egg laying of bivoltine races KA, NB18, multivoltine race PM and evolved races race-1 (R_1) and Race-2 (R_2) were selected.

Quantitative Analysis of Alpha and Beta Esterases

Esterase activity was determined following the procedure of Van Asperen using alpha- and beta-naphthyl acetate as substrates. The reaction mixture contained 5 ml of 0.3 m substrate prepared in 0.034 M phosphate buffer pH 7 and 1 ml of suitably diluted enzyme extract. After 30 minutes of incubation at 25°c, the reaction was arrested by adding 1 ml of colour reagent (a mixture of 0.1% diazo blue Band sodium lauryl sulphate in the ratio 2:5) optical density was measured by 600 nm using spectrophotometer. One unit of enzyme activity is defined as the amount of enzyme which produces one micro mole of naphthol/min under the above assay conditions (Van Asperen, 1962).

RESULTS AND DISCUSSION

Quantitative estimations of in Alpha- and Beta- esterases are expressed in terms of enzyme activity. This has been taken to evaluate the changes of enzyme activity in light of variations in the total protein concentration during different

developmental stages of races KA, NB_{18}, PM, R_1 and R_2. Each enzyme is present in a very low concentration in the eggs of all the species studied.

Esterase activity increases during larval instars and reaches a peak in pupae stage. It was found to be less in case of adults. The concentration was found to be high in KA followed by R_2, NB_{18}, R_1 and PM (Figures 1-8).

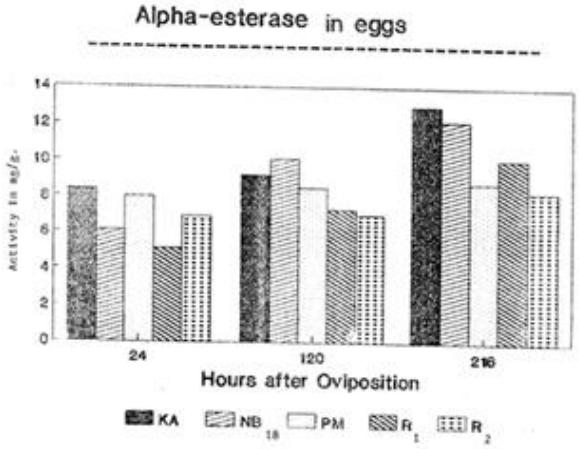


Figure 1

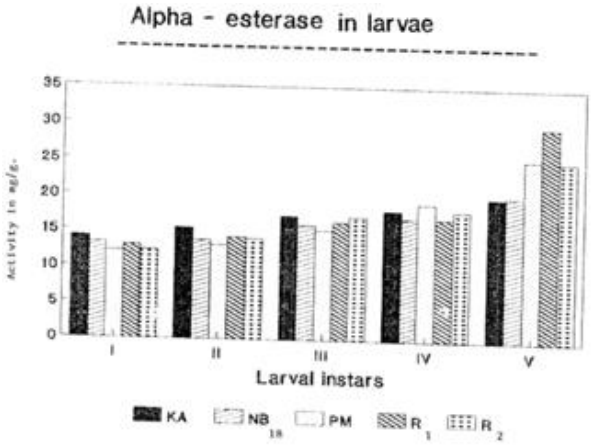
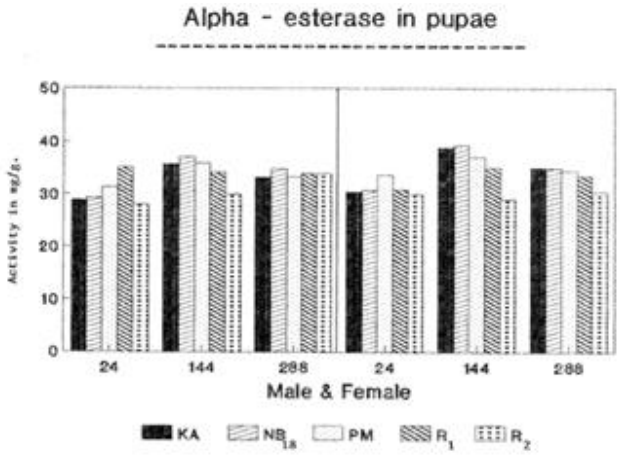


Figure 2



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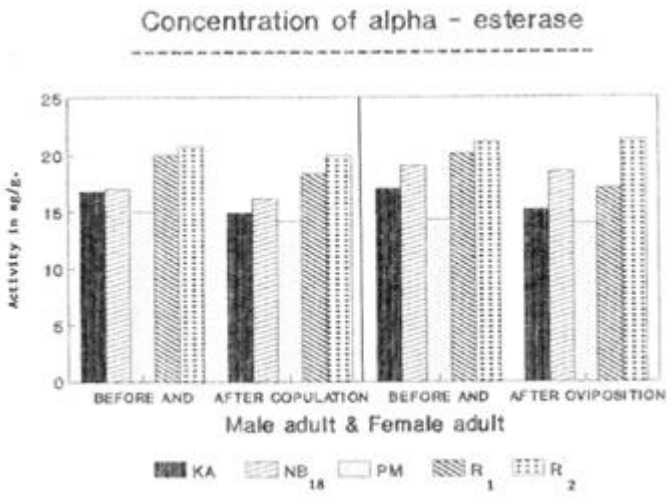

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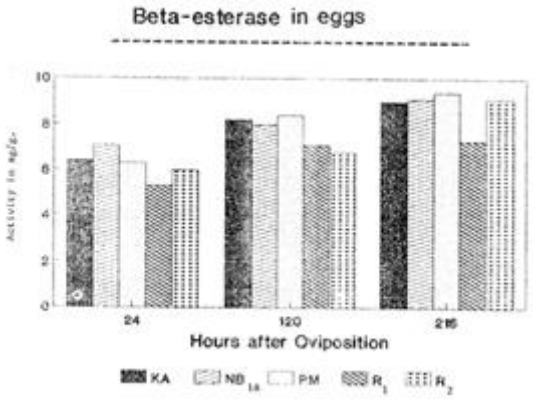

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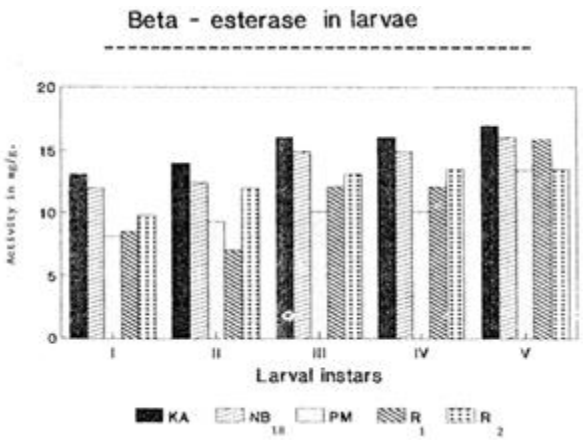

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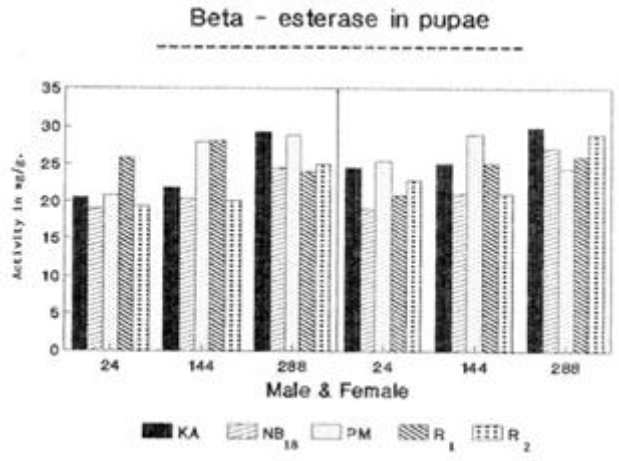

Figure 7

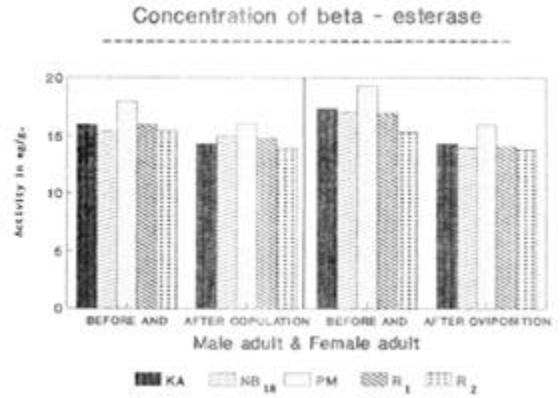

Figure 8

The results of the quantitative estimation of the alpha- and beta-esterase activity revealed that these esterases are increased from 24 hours' eggs pharate adult stage and in adults the activity was decreased. The activity was found to be high in pupae as reported by (Cannat, 1983). Comparatively the alpha-esterase activity is higher than beta-esterase. More or less same investigations have been conducted on the eri silkworm Philosamia ricini (Revanasiddaiah *et al.*, 1989). In a multivoltine strain of - mori (Pure Mysore) L- and beta- esterase isozyme patterns were reported by Krishnamurthy et al. But their studies were conducted around egg development and they noticed twelve isozymes in embryogenesis (Krishnamurthy *et al.*, 1984). However, in the present 14 and 10 isozymes in KA, 10 and 12 isozymes in NB_{18} and PM and 9 & 7 isozymes in R_1 and 6 bands in R_2 are recorded for L- and B- esterase respectively during embryogenesis. These findings were in accordance with the findings of Fei and Sheng wherein authors have reported a total of 12 esterase isozymes during embryogenesis (Fei and Sheng, 1987)

There is a gradual increase of esterase isozymes from eggs to pupae and they decreased in adults. This variation reflects regulation of gene activity so as to meet the den and of different metabolic activities (Revanasiddaiah *et al.*, 1989; Krishnamurthy *et al.*, 1984). The eggs show high esterase activity. This is due to the presence of large amounts of enzymes stored in the yolk which will be utilized during embryogenesis. The larval, pupal and adult stages reveal maximum heterogeneity like *P. ricini*. Esterase isozymes show sexual dimorphism in both pupal and adult stages. This shows stable difference in the expression of different genes in the same race of *Bombyx mori* during ontogeny.

The developmental esterases show a gradual increase in the number of isozymes from I larval to V larval instar of all the races studied including R_1 and R_2. Such an increase during development has been reported in Drosophila nastuta by Siddaveeregowda et al and (Siddaveeregowda *et al.*, 1977), also insects in general by Laufer (1961). A comparative study pertaining to larval developmental stages of the races show a gradual increase in the number of 1sozymes from I larval to V larval instar in all the races wherein very less number of bands are found in V instar larvae of PM. This may be due to the voltinism.

The results of the esterase activity in pupal stage of the five races studied indicate that alpha non-specific esterases are present in KA, NB_{18} and female pupae of PM and are absent in R_1 and R_2. However, high specific esterase activity is noticed in all the races. This observation coincides with that of Prakash and Reddy who have reported such high activity of alpha esterase in the pupal stage of fruit fly *Drosophila rajashekari* (Prakash and Reddy, 1978)

With reference to the beta specific esterases the activity is expressed only in PM and female pupae of R_1 the activity is expressed whereas it is absent in all the races but non-specific esterase activity is found in all the races. The functions of esterase isozymes in physiology of silkworm have been reported by many workers that esterases help in breaking down lipids and fatty acids (Kai and Hasegawa; 1972, Pant and Gupta, 1980; Oberlander and Chaeiderman, 1966; Kaur and Prakash, 1979). The specific esterases found in various developmental stages could be involved in modifying hormones responsible for subsequently metamorphic events growth, moulting, pupation and differentiation into adult (Kaur and Prakash, 1979).

CONCLUSION

In conclusion, results of our study delineated that alpha- and beta- esterases activity was found to be highest in the female pupae, and it was found to be less in case of adults. High esterase activity is noticed in pure races than the isolated lines. The esterase activity is high in pupal stage followed by larval stage.

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